PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PS216892-142	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/NZ2003/000059	International Filing Date *(day/month/year)* 7 April 2003	Priority Date *(day/month/year)* 5 April 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12N 5/00

Applicant

KIWI INGENUITY LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **8** sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [X] Certain observations on the international application

Date of submission of the demand 30 October 2003	Date of completion of the report 22 April 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer TERRY MOORE Telephone No. (02) 6283 2632

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000059

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-5, 7 and 11-53,** as originally filed,
page **6,** received on **23 January 2004** with the letter of **22 January 2004**
pages **8-10,** received on **3 March 2004** with the letter of **3 March 2004**

☒ the claims, pages **55-57, 59-61 and 64-66,** as originally filed,
pages , as amended (together with any statement) under Article 19,
pages **58, 62 and 63,** received on **3 March 2004** with the letter of **3 March 2004**
page **54,** received on **23 January 2004** with the letter of **22 January 2004**

☒ the drawings, pages **1-6,** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language,** all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-101	YES
	Claims		NO
Inventive step (IS)	Claims	1-101	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-101	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The present invention relates to a method of modifying an embryo by inserting an exogenous modified glycolipid into the embryo or zona pellucida membrane of the embryo, wherein the modification comprises incorporation of a binding moiety into the carbohydrate portion of the glycolipid and wherein the binding moiety enables binding of the glycolipid to an attachment molecule. Binding of the glycolipid to the attachment moiety facilitates association of the embryo with the cell membrane, in particular the endometrial cell membrane, and improves implantation.

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 WO 1999/005255
D2 Mol Hum Reprod 2, pp 52-9 (1996) Taylor et al
D3 J Reprod Fert 95, pp 813-823 (1992) Zhu et al

Novelty and Inventive Step

D1 discloses a method for improving implantation of embryos (see page 22). The method relies on insertion of a glycosylphosphatidylinositol (GPI)-linked exogenous protein into the membrane of an embryo and use of this to enhance implantation of the embryo. The GPI-linked proteins include adhesion proteins that attach to specific receptors and molecules on the uterine lining. However the GPI linkage is not a consequence of exogenous modification of the GPI to incorporate a binding part. As such the citation does not disclose or teach toward the claimed subject matter.

D2 discloses GPI-anchored complement-binding proteins that are strongly expressed by human preimplantation blastocysts and cumulus cells. The (GPI)-anchored proteins are expressed by the plasma membrane and zona pellucida of oocytes, embryos and expanded preimplantation blastocysts. However the citation does not disclose or teach toward modification of the GPI anchor or use of such a construct to improve implantation. As such the citation does not appear to deprive the claims of novelty or an inventive step.

Continued in supplemental box.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-3, 5-33, 34-70 and 72-101 are not fully supported by the extent of disclosure in the specification. The specification discloses modification of glycolipids through attachment of non-specific binding moieties such as biotin or a chelator to the carbohydrate portion of the glycolipid. As such the specification provides support for attaching a range of binding moieties to the portion of a glycolipid that is expressed at the surface of the membrane such that the lipid tail is free to insert in the membrane, thereby locating the binding moiety on the surface of the embryo or zona pellucida membrane.

In contrast the claims are silent with respect to the location of the binding partner on the glycolipid membrane or the means of attachment. As such the claims include within their scope methods of attachment and action that do not rely on the principles disclosed in the specification and that could not be routinely achieved based on the extent of the information in the specification.

In their response the attorney suggested that the claims need not be limited to attachment to the carbohydrate portion of the glycolipid because it was "*within the scope of the invention for the modification to be to a portion of the glycolipid molecule that is expressed at the surface of the membrane in to which the glycolipid is inserted*". The examiner accepts this but it still remains that the invention requires that the glycolipid is modified in such a way that the lipid tail can still insert normally into the membrane and that the binding moiety is located at the membrane surface. The only means for achieving this that are provided in the specification are those means associated with attachment of the binding moiety to the portion of the glycolipid that is located at the surface of the membrane. As such the feature is essential to the claims.

Claims 1-101 all relate to embryos and biological methods of generating human beings. As such the claims may not represent patentable subject matter in many patent states.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V2

D3 discloses and teaches that carbohydrate chains of cell surface glycolipids may play a crucial role in regulating maternal-foetal interactions during implantation and early development. It further discloses that rapid changes in the glycolipid composition of endometrial cells during early pregnancy may facilitate embyro adhesion and trophectoderm outgrowth during implantation. However, as with D2, the citation does not disclose or teach toward modification of the GPI anchor or use of such a construct to improve implantation. As such the citation does not appear to be relevant to the novelty or inventive step of the claims.

It is an object of this invention to provide a modified embryo for the enhanced implantation of the embryo into the endometrium of an animal, or to at least provide the public with a useful choice.

STATEMENTS OF INVENTION

In a first aspect of the invention there is provided a glycolipid-inserted embryo for the preparation of an embryo modified for enhancing the implantation of the embryo into the endometrium of an animal, where:

- the glycolipid-inserted-embryo has an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
- the glycolipid has been modified to incorporate a binding part wherein said binding part is adapted to enable binding to an attachment molecule.

Preferably, the glycolipid has been modified to incorporate the binding part prior to the insertion of its lipid tails into the cell membranes of the embryo or into the zona pellucida of the embryo.

In a second aspect of the invention there is provided an embryo modified for enhancing the implantation of the embryo into the endometrium of an animal, where:

- the embryo has an attachment molecule which is capable of attaching to the endometrium; and
- the attachment molecule is linked to the embryo by an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
- the attachment molecule and the glycolipid have each been modified to incorporate a binding part adapted to enable the attachment molecule and the glycolipid to be bound together via their respective binding parts either directly or through a bridging molecule.

The glycolipid may be any glycolipid capable of inserting its lipid tails into the cell membranes of the embryo or into the zona pellucida of the embryo such as phosphoglycerides or sphingolipids. The glycolipid may be a natural molecule or a modified (e.g. biotinylated) glycolipid. Preferably the modified glycolipid is a biotinylated glycolipid either of the ganglioside class that contains sialic acid groups, or the neutral class that contains galactose.

The attachment molecule may be any molecule that has a binding affinity for molecules on cell membranes (e.g. receptor sites and blood group related antigens) including their mucus coat. Preferably the cell membrane is endometrial. In particular, the attachment molecule is preferably a protein, a peptide (such as poly L-lysine) a carbohydrate, an acyl group, a polymer, or an immunoglobulin such as immunoglobulin G (IgG) or a lectin. Alternatively, the attachment molecule may be a synthetic molecule (e.g. polyvinyl pyrrolidine, or an acyl group) which reacts with molecules expressed on cell membranes or on the mucus layer covering the cell membrane. The attachment molecule can itself be a glycolipid or glycolipid conjugate.

In a third aspect of the invention there is provided a method of preparing the glycolipid-inserted-embryo of the first aspect of the invention including the step:

- contacting a glycolipid with an embryo, where the glycolipid has been exogenously modified to incorporate a binding part, wherein said binding part is adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo.

In a fourth aspect of the invention there is provided a method of preparing the modified embryo of the second aspect of the invention including the steps:

- contacting an attachment molecule with a glycolipid, where the attachment molecule and the glycolipid have each been modified to incorporate a binding part adapted to enable the attachment molecule and the glycolipid to bind together via their respective binding parts either

directly or through a bridging molecule to provide a glycolipid-attachment molecule construct; and then

- contacting the attachment molecule bound to the glycolipid (glycolipid-attachment molecule construct) with an embryo so that the lipid tails of the glycolipid insert into the cell membranes of the embryo or into the zona pellucida of the embryo:

Or including the steps:

- contacting a glycolipid with an embryo, where the glycolipid has been exogenously modified to incorporate a binding part adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo; and then
- contacting the glycolipid-inserted-embryo with an attachment molecule, modified to incorporate a binding part wherein said binding part is adapted to enable binding to the binding part of the glycolipid either directly or through a bridging molecule.

Preferably the glycolipid has been modified to incorporate a binding part comprising biotin and the attachment molecule has been modified to incorporate a binding part comprising avidin.

Alternatively, the glycolipid has been modified to incorporate a binding part comprising avidin and the attachment molecule has been modified to incorporate a binding part comprising biotin.

In the case of binding of the glycolipid to the attachment molecule through a bridging molecule, it is preferred that the bridging molecule comprises avidin and that both the glycolipid and the attachment molecule have been modified to incorporate binding parts comprising biotin.

In a fifth aspect of the invention there is provided a method of enhancing the implantation of an embryo into the endometrium of an animal, preferably a human, or domesticated animal, comprising the steps:

- preparing a modified embryo according to the second aspect of this invention, and
- transferring the modified embryo to the uterus of the animal.

In one embodiment of the invention the modified embryo is prepared from a species, hybrid or variety of animal that is the same as the species, hybrid or variety of animal, to the uterus of which it is transferred. In an alternative embodiment, the species, hybrid or variety differ.

In a sixth aspect of the invention there is provided a glycolipid-attachment molecule construct when used for generating a modified embryo comprising a glycolipid modified to incorporate a binding part and an attachment molecule modified to incorporate a binding part wherein the respective binding parts are adapted to enable the modified glycolipid and the modified attachment molecule to bind each other either directly or indirectly through a bridging molecule.

In a seventh aspect of the invention there is provided a method of enhancing the implantation of an embryo into the endometrium of an animal including the steps of:

- introducing a construct of the sixth aspect of the invention into the uterus of the animal so that the construct becomes localised to the endometrium; and then
- transferring the embryo to the uterus of the animal.

In an eighth aspect the invention provides a kit for use in enhancing the implantation of an embryo of an animal comprising one or more preparations of a glycolipid-attachment molecule construct of the sixth aspect of the invention.

While the invention is broadly defined as above, those persons skilled in the art will appreciate that it is not limited thereto and that it also includes embodiments of which the following description provides examples. In addition, the present invention will be better understood from reference to the figures of the accompanying drawings.

32. An embryo as claimed in claim 31 wherein the molecules on cell membranes are receptor sites and/or blood group related antigens.

33. An embryo as claimed in claim 31 or claim 32 wherein the cell membranes are endometrial cell membranes.

34. A method of preparing a glycolipid-inserted-embryo including the step of:

- contacting a glycolipid with an embryo, where the glycolipid has been exogenously modified to incorporate a binding part, wherein said binding part is adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the modified glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo.

35. A method of preparing a modified embryo including the steps of:

- contacting an attachment molecule with a glycolipid, wherein the attachment molecule and the glycolipid have each been modified to incorporate a binding part adapted to enable the attachment molecule and the glycolipid to bind together via their respective binding parts either directly or through a bridging molecule; and then
- contacting the attachment molecule bound to the glycolipid with an embryo so that the lipid tails of the glycolipid insert into the cell membranes of the embryo or into the zona pellucida of the embryo.

36. A method of preparing a modified embryo including the steps:

- contacting a glycolipid with an embryo wherein the glycolipid has been exogenously modified to incorporate a binding part, wherein said binding part is adapted to enable binding to an attachment molecule either directly or through a bridging molecule, so that the lipid tails of the glycolipid insert into a cell membrane of the embryo or into the zona pellucida of the embryo to provide a glycolipid-inserted-embryo; and then

63. A method as claimed in any one of claims 34 to 62 wherein the attachment molecule is a molecule that has a binding affinity for molecules on cell membranes including the mucus coat of cell membranes.

64. A method as claimed in claim 63 wherein the molecules on cell membranes are receptor sites and/or blood group related antigens.

65. A method as claimed in claim 63 or claim 64 wherein the cell membranes are endometrial.

66. A method of enhancing the implantation of an embryo into the endometrium of an animal including the steps:

- preparing a modified embryo according to the method of any one of claims 35 to 65; and
- transferring the modified embryo to the uterus of the animal.

67. A method as claimed in claim 66 including the step:

- introducing a component with which the attachment molecule will interact into the uterus of the animal so that the component becomes localised to the endometrium.

68. A method as claimed in claim 66 or claim 67 wherein the animal is a human or domesticated animal.

69. A method as claimed in claim 66 or claim 67 wherein the modified embryo is prepared from a species, hybrid or variety of animal different from the species, hybrid or variety of animal of the uterus.

70. A glycolipid-attachment molecule construct when used for generating a modified embryo comprising a glycolipid modified to incorporate a binding part and

an attachment molecule modified to incorporate a binding part wherein the respective binding parts are adapted to enable the modified glycolipid and the modified attachment molecule to bind to each other either directly or indirectly through a bridging molecule.

71. A construct as claimed in any one of claims 70 wherein the modification to the glycolipid is to the carbohydrate portion of the glycolipid.

72. A construct as claimed in claims 70 or 71 wherein the attachment molecule is selected from the group consisting of carbohydrates or oligosaccharides, glycolipids, glycoconjugates, proteins, peptides, acyl groups or polymers.

73. A construct as claimed in any one of claims 70 to 72 wherein the attachment molecule is selected from the group consisting of natural or synthetic carbohydrates or oligosaccharides, proteins or peptides including poly L-lysine, antibodies, lectins, polyvinyl pyrrolidine, and functionally equivalent derivatives thereof.

74. A construct as claimed in any one of claims 70 to 73 wherein the attachment molecule is an immunoglobulin.

75. A construct as claimed in claim 74 wherein the attachment molecule is immunoglobulin G (IgG).

76. A construct as claimed in any one of claims 70 to 75 wherein the attachment molecule is adapted to interact with the epithelial cells of the endometrium, mucus, mucin, or other endogenous or exogenously provided component of mucus.

77. A construct as claimed in any one of claims 70 to 76 wherein the attachment molecule is an endometrial attachment molecule.

Received 23 January 2004

CLAIMS

What is claimed:

1. A glycolipid-inserted-embryo for the preparation of an embryo modified to enhance the implantation of the embryo into the endometrium wherein:

- the glycolipid-inserted-embryo has an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
- the glycolipid has been modified to incorporate a binding part wherein said binding part is adapted to enable binding to an attachment molecule.

2. An embryo as claimed in claim 1 wherein the glycolipid has been modified to incorporate the binding part prior to the insertion of the lipid tails into a cell membrane of the embryo or into the zona pellucida of the embryo.

3. An embryo modified to enhance the implantation of the embryo into the endometrium wherein:

- the embryo has an attachment molecule which is capable of attaching to the endometrium; and
- the attachment molecule is linked to the embryo by an exogenously modified glycolipid having lipid tails inserted into a cell membrane of the embryo or into the zona pellucida of the embryo; and
- the attachment molecule and the glycolipid have each been modified to incorporate a binding part so that the attachment molecule and the glycolipid are bound together via their respective binding parts either directly or through a bridging molecule.

4. An embryo as claimed in any one of claims 1 to 3 wherein the modification to the glycolipid is to the carbohydrate portion of the glycolipid.